SHORT COMMUNICATION

Effect of humic acid on yield and oil characteristics of *Silybum marianum* and *Cucurbita pepo* convar. *pepo* var. *styriaca* seeds

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S u m m a r y

Extreme use of chemical fertilizers could reduce the quality of secondary metabolites in medicinal plants. Humic acid (HA) might benefit plant growth by improving nutrient uptake and hormonal effects. The main objective of this work was to study the effect of HA on yield of *Silybum marianum* (milk thistle) and *Cucurbita pepo* convar. *pepo* var. *styriaca* (medicinal pumpkin) seed and the seed oil characteristics. Different levels of HA (0, 500 and 1000 mg/L) were applied weekly on plants as foliar application one month after seed germination. The results showed that HA had no effect on yield of milk thistle and decreased pumpkin yield. HA treatment decreased oil content of both species. Regarding to oil quality, gas chromatography results indicated that oils unsaturation degree was improved by HA application. It could be concluded that although HA had no positive effect on yield and oil content, it was worthy in enhancement of nutritional value of the oils.

Key words: *humic substances, milk thistle, medicinal pumpkin, yield, oil quality*

INTRODUCTION

Humic acid (HA) is a promising natural resource to be used as an alternative for fertilizers to increase crop production [1]. It is a natural polymeric organic compound produced by the decay of organic materials and found in soil, peat and lignites [2]. The major functional groups of HA include carboxyls, phenolic hydroxyls, alcoholic hydroxyls and ketones [3]. Presently, humic preparations from leonardite are available for use in agriculture. It has been reported that humic substances (HS) have beneficial effects on plant growth, nutrient uptake, root development, yield, seed germination and plant photosynthesis [4, 5]. As a consequence, the use of HS is proposed as a method of improvement of crop production.

HS are usually applied to the soil and favorably affect the soil structure and microbial populations [4]. Foliar spray of these substances also promotes growth in a number of plant species such as tomato, cotton and olive [6]. Several hypotheses have been proposed to explain the effect of HA. These include the formation of a complex between HA and mineral ions, influence of HA on respiration and photosynthesis and stimulation of nucleic acid metabolism and hormone-like activity of HA [5].

Medicinal pumpkin and milk thistle have been the subject of some studies in recent years. The seeds of these species contain some active substances widely used in traditional and industrial pharmacology [7, 8, 9]. Silymarin, derived from the milk thistle, has been used for centuries as a natural remedy in the treatment of hepatitis and cirrhosis, as well as in the protection of the liver from toxic substances [10, 11]. Recent reports have demonstrated that silymarin has also exceptionally high antitumor promoting activity [11, 12]. Seeds of medicinal pumpkin are rich in polyunsaturated fatty acids which not only help in preventing arteriosclerosis, high blood pressure and heart diseases, but also stimulate the metabolism of accumulated fats [13]. However, it should be considered that extreme use of chemical fertilizers can reduce the quality of secondary products in medicinal plants. Since there are no reports on the influence of HA on these species, the objective of this study was to evaluate the effects of foliar application of HA on plant yield and seed oil characteristics of milk thistle and medicinal pumpkin.

MATERIAL AND METHODS

Cultural practice

The research was carried out in spring and summer 2009 in the field of Horticulture Department, Isfahan University of Technology, Iran. A randomized block design with three replications was carried out to evaluate the effect of different levels of HA (0, 500 and 1000 mg/l) on medicinal pumpkin and milk thistle. First, HA was dissolved in distilled water and then was applied weekly as foliar application one month after seed germination. The plants were cultured directly by seed. The medicinal pumpkin seeds were primed 24 hrs in distilled water before culture. HA was extracted from leonardite and contained carbon (C), 61.2%; nitrogen (N), 3.13 g/kg and phosphorus (P), 2.89 g/kg dry matter purchased in China. HA was sprayed on all plant surfaces (using a hand-held sprayer) at early morning hours until the leaves were completely wet. All plants were fertilized once with Kemira 10:10:10 fertilizer (Yara Co., Norway) one month after sowing at the rate of 100 kg N/ha.

The plants were grown in three row plots, with five meters long. The plants spacing was 30 cm × 50 cm for milk thistle and 50 cm × 200 cm for medicinal pumpkin. The soil mixture used in the study had a sandy-loam texture pH 7.2 and electrical conductivity 2.5 dS/m. It contained 810 ppm N, 15 ppm P and 100 ppm K. During the growing period, plants were furrow irrigated and weeds were controlled by hand as needed. The plant material including leaves and seeds ware collected at the end of experiment for further analysis (Sep. 2009).

Oil content in seed

Seed oil was extracted using the Soxhlet extraction apparatus and petroleum ether (40–60°C) as a solvent. As 20 g of oil was needed for further analysis, 60 g of pumpkin seeds and 100 g of milk thistle seeds were taken for oil extraction. The extracted oil was separated from the organic solvent using a rotary vacuum evaporator. To avoid changes in the chemical composition of samples, they were frozen immediately after extraction and stored under a nitrogen atmosphere at -18°C.

Physicochemical properties of oil

It was appeared that HA treatment might be effective on the synthesis pathway of color compounds such as chlorophyll and carotenoides in the plant and changes the content of these pigments in the oil. Therefore, in this work, oil color was measured as a physical parameter of the oil. Oil color was determined by the method of AOCS Cc 13e-92 applying Lovibond Tintometer (PFX 995, South Africa) using a glass cell with an optical path length of 1 in. This method determines the color by matching the color of the light transmitted through a specific depth of liquid oil to the color of the light originating from the same source, transmitted through glass color standards. The refractive index of the oil was evaluated using a digital hand refractometer (DR-201-95, Germany) in daylight calibrated against pure water at 25°C.

Specific gravity of the oil was determined by Pycnometer method, AOCS Cc10a-25 and reported at 20/20°C [14]. The iodine value was measured by the method of Cd 1-25 AOCS [14].

Fatty acids composition of oil

Fatty acids compositionof was determined by gas liquid chromatography (GC). The methyl esters were prepared using the method described by Goli et al. [15]. One hundred microliters of sodium methoxide (0.5 M) was added to 50 μl sample in 1 ml of n-hexane. The mixture was shaken vigorously for 15 min, allowed to stand and separate. Hexane phase was separated and 1 μl was injected to GC at a split ratio of 20:1. The gas chromatographic analysis of FAME was performed on an Agilent 6890N gas chromatograph equipped with a flame ionization detector. The column used was a HP-88 (100 m, 0.25 mm i.d., 0.2 μm film thickness) column. The temperature program consisted of increasing the temperature first from 150ºC to 210ºC at a rate of 5ºC/min and holding for 8 min, then increasing to 240ºC at a rate of 5ºC/min and holding for 6 min. Temperatures of injector and detector were 230 and 250ºC, respectively. High-purity nitrogen was used as a carrier gas.

All solvents/chemicals used were of analytical grade and obtained from Merck (Darmstadt, Germany). Pure fatty acids, including palmitic, stearic, oleic, linoleic, and linolenic acids were purchased as standards from the Sigma Chemical Co. (St Louis, MO).

Statistical analysis

The experiment was set up as a randomized complete block design with three replications of each treatment. All data were subjected to a one-way analysis of variance (ANOVA), followed by the LSD comparison of means using procedure within the SAS and Statistix systems.

RESULTS AND DISSCUSION

The results showed that HA application did not affect milk thistle yield (tab. 1). Regarding to pumpkin, yield decreased in higher concentration of HA compared to the control by 53.12%. Similar results were reported on grape [16] and olive [6]. These researches reported that foliar application of HA might not be effective in all plants. Most reports on application of HS regard to the soil applications. In soil application HS affect yield mostly through improving root development and, as a consequence, nutrients uptake enhancement [1, 4]. On the other hand HS can improve stress resistance, including drought resistance in some species such as creeping bentgrass, through its hormonal activities [17]. Probably the plants used in the present study did not suffer from any stresses. Therefore, foliar application of HA could not improve the plant performance.

Table 1.

Effect of humic acid on yield (total seed weight) of milk thistle and pumpkin

Humic acid (mg/l)	Yield (g/m^2)	
	Milk thistle	Pumpkin
0	21.87^A	51.58^A
500	24.18^A	44.12^A
1000	21.23^A	24.18^B

In columns the means with the same letters are not significantly different at 5% LSD test.

The effects of HA treatments on oil content and quality characteristics are summarized in table 2. In terms of oil percentage, higher HA concentration significantly decreased oil content in milk thistle and pumpkin seeds ($p<0.05$). Only one report is available concerning this case. It indicates that HA foliar application had no effect on oil percentage in olive fruits [6]. Regarding to the oil quality, data presented in table 2 indicated that HA had a positive effect on refractive index (RI) of oils. In both seed oils, RI increased in parallel with HA concentration, even though in milk thistle this enhancement was insignificant. Iodine value (IV) of pumpkin oil as well as RI increased by higher HA concentration which confirmed RI result. This value in milk thistle oil increased by 500 mg/l HA (114.16) but decreased in 1000 mg/l (113.85) which was not significant. Both IV and RI are indicators of unsaturation degree of oils and higher IV and RI imply higher degree of unsaturation. The results showed that HA treatments made higher unsaturation degree in oils specifically in pumpkin whereas milk thistle demonstrated a reduction of unsaturation degree in 1000 compared to 500 mg/l HA. Specific gravity (SG) of oils depends on chain length and unsaturation degree of fatty acids. Higher chain length as well as higher degree of unsaturation lead to higher SG [18]. As expected, pumpkin oil SG was increased (from 0.881 to 0.886) while this value was decreased in milk thistle oil (from 0.903 to 0.885) by addition of 1000 mg/l HA, which confirmed the results of IV and RI.

Table 2.

Effect of humic acid on oil content and quality of milk thistle and pumpkin seeds

Humic acid (mg/l)	Oil content (%)		Refractive index (25°c)		Specific gravity (20/20°c)		Iodine value (g i_2/100 g)	
	Milk thistle	Pumpkin	Milk thistle	Pumpkin	Milk thistle	Pumpkin	Milk thistle	Pumpkin
0	17.40^a	36.38^a	1.4650^a	1.4570^b	0.903^a	0.881^b	110.86^b	94.54^b
500	14.81^b	31.80^b	1.4665^a	1.4635^a	0.883^b	0.885^{ab}	114.16^a	96.16^a
1000	15.45^b	33.18^b	1.4663^a	1.4630^a	0.885^b	0.886^a	113.85^a	96.09^a

In columns the means with the same letters are not significantly different at 5% LSD test.

Table 3 presents the effect of HA treatments on oil color. Lovibond method using color glasses is the accepted international standard for the measurement of color in animal and vegetable fats and oils. Using Lovibond instrument, oil color can be determined and classified based on three important color scales of red, yellow and blue. Oil color, as a qualitative factor, indicates whether the oil needs bleaching process in oil refinery or not [18]. As shown, pumpkin oil (control) had two hues of red (68.0) and blue (13.4) but as long as HA was applied, blue color intensity increased (19.5) whereas red intensity was constant. Milk thistle oil had red (3.4) and yellow (70.0) as two dominant hues. By increasing HA concentration, color intensity of red (4.0) and yellow (72.0) increased. In conclusion, the results showed that HA might be of very low effect on the enhancement of color intensity in oils.

Table 3.

Effect of humic acid on oil color of milk thistle and pumpkin seeds

Humic acid (mg/l)	Blue		Yellow		Red	
	Milk thistle	Pumpkin	Milk thistle	Pumpkin	Milk thistle	Pumpkin
0	0	16	70	0	3.9	68
500	0	16	70	0	3.9	68
1000	0	19.5	72	0	4	68

Table 4.

Effect of humic acid on fatty acids profile (%) on milk thistle and pumpkin oils

Fatty acid	Palmitic		Stearic		Oleic		Linoleic		Linolenic		Arachidic	
Humic acid (mg/l)	Milk thistle	Pump-kin	Milk thistle	Pump-kin	Milk thistle	Pump-kin	Milk thistle	Pump-kin	Milk thistle	Pump-kin	Milk thistle	Pump-kin
0	11.01	14.02	6.30	7.83	36.57	45.70	41.82	30.98	2.67	0.59	1.62	0.40
500	9.30	13.88	6.34	7.50	35.77	45.33	42.90	32.40	3.48	0.41	2.21	0.48
1000	8.94	13.07	6.45	7.20	36.58	47.95	42.22	31.03	3.55	0.43	2.25	0.32

Fatty acid composition of oils during HA treatment is represented in table 4. The fatty acid analysis of the extracted pumpkin seed oil indicated that oleic (45.70%), linoleic (30.98%) and palmitic (14.02%) acids were predominant fatty acids. While palmitic acid content decreased, two unsaturated fatty acids including oleic and linoleic acids increased by HA treatments. In consequence this enhancement led to a higher oil unsaturation degree. Similarly, main fatty acids in milk thistle oil were linoleic (41.82%), oleic (36.57%) and palmitic (11.01%) acids, respectively (tab. 4). In both species, HA application resulted in increasing of oil unsaturation degree. It might be implied that HA was probably effective on synthesis cycle of fatty acids in plant and could improve unsaturated fatty acids content.

CONCLUSION

HA did not positively influence the yield of both species and seed oil content was correlated negatively to HA treatments. In terms of oil characteristics, HA was effective on fatty acids composition and increased unsaturation degree of oils leading to higher IV and RI indices. However, more studies might be needed to clear the effect of HS on synthesis cycle of main components synthesis cycle such as fatty acids.

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WPŁYW KWASÓW PRÓCHNICZYCH NA PLON I CECHY OLEJU W NASIONACH SILYBUM MARIANUM I *CUCURBITA PEPO* CONVAR. *PEPO* VAR. *STYRIACA*

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S t r e s z c z e n i e

Stosowanie dużych ilości nawozów sztucznych może obniżyć jakość wtórnych produktów przemiany materii w roślinach leczniczych. Kwasy próchnicze (humic acids, HA) mogą polepszyć wzrost rośliny poprzez poprawę przyswajania składników odżywczych i działanie hormonopodobne. Głównym celem tej pracy była analiza wpływu kwasów próchniczych na plon nasion ostropestu plamistego (*Silybum marianum*) i dyni zwyczajnej, odmiana *styriaca* oraz charakterystykę oleju. Różne stężenia HA (0, 500 i 1000 ml/l) podawano dolistnie co tydzień roślinom miesiąc po wykiełkowaniu nasion. Wyniki pokazały, że HA nie wpływały na plon ostropestu plamistego i plon dyni. HA obniżyły też zawartość oleju w obu badanych gatunkach. Wyniki chromatografii gazowej dotyczące jakości oleju wskazują, że stopień nienasycenia olejów zwiększył się po zastosowaniu kwasów HA. Można podsumować, że chociaż kwasy próchniczne nie wpływają pozytywnie na plon i zawartość oleju, to mają znaczenie dla zwiększenia wartości odżywczej oleju.

Słowa kluczowe: *kwasy próchnicze, ostropest plamisty, dynia lecznicza, plon, jakość oleju*